EVALUATION OF ANTI-TUBERCULOSIS POTENTIALS OF SELECTED MEDICINAL PLANTS IN ENDAU ROMPIN, JOHOR, MALAYSIA

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DEDICATION

The thesis is first and foremost dedicated to Almighty Allah for seeing me through. Then to my parents Alhaji Sanusi B. Mohammad and Hajiya Binta Sanusi for their unwavering support, advice, encouragement and prayers which guided me towards this achievement, I am very proud of them and may Almighty Allah (S.W.T) reward them abundantly. The thesis is also dedicated to my wife, children, siblings, uncles and aunties for their prayers and support.

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ABSTRACT

Tuberculosis (TB) remains an escalating health crisis globally which prompts new approaches to find more effective therapeutic strategies. Medicinal plants of Malaysia have a significant role to play in being able to provide new therapeutic remedies. The local people of Kampung Peta (Jakun tribe), Endau Rompin claimed that local preparations of some plants are used to treat symptoms of tuberculosis. There is a need to validate the claim by tradition healers scientifically. The aim of this research is to search for anti-TB from plants of Taman Negara Johor Endau-Rompin, exploiting the traditional medical practices of the Jakun people. Aqueous and organic extracts of these plant species were screened for their antimycobacterial activity using agar disk diffusion assay, Tetrazolium Microplate Assay and agar plate assay against *Mycobacterium smegmatis*. The effect of the extract on mycobacterial cell at the cellular level was investigated upon treatment with the crude extracts via time-kill analysis, leakage of compound absorbing at 280nm, and field emission-scanning electron microscopy (FE-SEM). The findings revealed that methanol extract of *Nepenthes ampularia* displayed the largest zone of inhibition (DIZ=18.67 ± 0.58 mm). Ethyl acetate extract of *Musa gracilis* and hexane extract of *N. ampularia* exhibited the lowest minimum inhibitory concentration (MIC=0.39 mg/mL). Hexane extract of *N. ampularia* showed the lowest minimum bactericidal concentration (MBC= 1.56 mg/mL). At 3-fold of MIC, hexane extract of *N. ampularia*, ethyl acetate extract of *M. gracilis* and ethyl acetate extract of *N. ampularia* killed the entire bacterial cell within 8 h of exposure by causing the cell lysis. The GC-MS analysis revealed the presence of phytoconstituents that might contribute to the antimycobacterial effect. The study scientifically justified the use of the selected medicinal plant species by Jakun people. Further studies on *N. ampularia* and *M. gracilis* could lead to the development of new anti-TB drugs.

ABSTRAK

Tuberkulosis (TB) atau batuk kering masih merupakan krisis kesihatan sejagat yang terus meningkat sehingga menyebabkan para penyelidik cuba mencari pendekatan baru untuk menemui strategi terapeutik yang lebih berkesan. Masyarakat Kampung Peta (etnik Jakun), Endau Rompin mendakwa bahawa beberapa ramuan daripada tumbuhan tempatan telah digunakan dalam perawatan simptom TB. Tujuan kajian ini ialah mencari bahan anti-TB daripada sumber tumbuhan di Taman Negara Johor Endau Rompin, serta mengeksploitasi amalan amalan tradisional masyarakat Jakun. Tumbuhan terpilih telah diselidiki bagi mendapatkan bahan anti-TB yang berpotensi terhadap *Mycobacterium smegmatis*. Ekstrak akuas dan organik tumbuhan ini telah diskrin/disaring bagi aktiviti-aktiviti anti-mikobakteria menggunakan asai cakera serapan (Tetrazolium Microplate Assay), dan asai plat agar terhadap *M. smegmatis*. Kesan ekstrak pada sel mikobakteri di peringkat sel telah disiasat apabila rawatan dengan ekstrak mentah melalui analisis tempoh-mati, ketirisan sebatian yang diserap pada 280nm, dan mikroskopi elektron pengimbas-emisi lapangan. Dapatan menunjukkan bahawa ekstrak metanol *Nepenthes ampularia* mempamerkan zon perencatan yang lebih besar/lebar (DIZ=18.67 ± 0.58 mm). Ekstrak etil-asetat *Musa gracilis* dan ekstrak heksana *N. ampularia* mempamerkan kepekatan perencatan minimum terendah (MIC=0.39 mg/mL). Ekstrak heksana *N. ampularia* menunjukkan kepekatan bakterisidal minimum terendah (MBC= 1.56 mg/mL). Pada kepekatan 3 kali ganda MIC, ekstrak heksana *N. ampularia*, ekstrak etil asetat *M. gracilis* dan ekstrak etil asetat *N. ampularia* mematikan keseluruhan sel bakteria dalam tempoh pendedahan 8 jam, dengan cara lisis sel. Hasil analisis GC-MS menunjukkan kehadiran fitokonstituen yang mungkin menyumbang kepada kesan anti-mikobakterial. Kajian ini secara saintifik telah menjustifikasi penggunaan tumbuhan terpilih di kalangan suku etnik Jakun. Kajian lanjutan terhadap *N. ampularia* and *M. gracilis* berkemungkinan akan dapat membantu dalam membangunkan dadah anti TB yang baru.

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LIST OF SYMBOLS AND ABBREVIATIONS

<	-	less than
%	-	percentage
˚C	-	degree celcius
>	-	greater than
≤	-	less than or equal to
≥	-	greater than or equal to
µg	-	microgram
µl	-	microliter
µm	-	micrometre
1X	-	1 fold of
2X	-	2 fold of
3X	-	3 fold of
ADC	-	Albumin Dextrose Catalase
AIDS	-	Acquired Immunodeficiency Syndrome
ATCC	-	American Type Culture Collection
BCG	-	Bacille Calmette Gue´rin
BL-3	-	biosafety level 3
CFU	-	Colony Forming Unit/mL
cm	-	Centimetres
CNS	-	Central Nervous System
DIZ	-	Diameter of inhibition zone
DMSO	-	Dimethyl sulphoxide
DNA	-	Deoxyribonucleic acid
DOTS	-	Directly Observed Treatment – Short course
EMB	-	Ethambutol
EMGR	-	Ethyl acetate extract of *Musa gracilis*
ENA	-	Ethyl acetate extract of *Nepenthes ampularia*

EtOH	-	Ethanol
FE-SEM	-	Field Emission Scanning Electron Microscope
GC-MS	-	Gas chromatography–mass spectrometry
GI	-	Gastrointestinal
H	-	Hour
HIV	-	Human Immunodeficiency Virus
HNA	-	Hexane extract of *Nepenthes ampularia*
HPLC	-	High Performance Liquid Chromatography
IM	-	Intramuscular
INH	-	Isoniazid
IV	-	Intravenous
JNPC	-	Johor National Park Corporation
MABA	-	Microplate Alamar Blue Assay
MBC	-	Minimum Bactericidal Concentration
MCA	-	Methanol extract of *Campnosperma auriculatum*
MDR	-	Multidrug-Resistant
mg	-	milligram
MIC	-	Minimum Inhibitory Concentration
min	-	minute
mL	-	Millilitre
mm	-	millimetre
NA	-	Not active
NIST	-	National Institute of Standard and Technology
nm	-	nanometres
OADC	-	Oleic acid, Albumin, Dextrose and Catalase
OD	-	Optical density
ORAC	-	Oxygen radical absorbance capacity
PAS	-	Para-Amino Salicylic acid
PYR	-	Pyrazinamide
RIF	-	Rifampin
RNA	-	Ribonucleic acid
rpm	-	Revolution per minute
RT	-	Retention time

SD	-	Standard deviation
STR	-	Streptomycin
TB	-	Tuberculosis
TDM	-	Trehalose dimycolate
TEMA	-	Tetrazolium microplate assay
TLC	-	Thin-Layer Chromatography
TLR	-	Toll like receptors
TMM	-	Trehalose monomycolate
TNJER	-	Taman Negara Johor Endau-Rompin
UTHM	-	Universiti Tun Hussein Onn Malaysia
WHO	-	World Health Organization
XDR-TB	-	Extensive drug-resistant

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LIST OF PUBLICATIONS

Journals:

(i) **Sanusi, S. B.,** Bakar, A., Fadzelly, M., Mohamed, M., Sabran, S. F., & Mainasara, M. M. (2017). Southeast Asian Medicinal Plants as a Potential Source of Antituberculosis Agent. *Evidence-Based Complementary and Alternative Medicine, 2017*. https://doi.org/10.1155/2017/7185649

(ii) **Sanusi, S. B.,** Bakar, M. F. A., Mohamed, M., Sabran, S. F., & Mainasara, M. M. (2017). Ethnobotanical, phytochemical, and pharmacological properties of nepenthes species: A review. *Asian J Pharm Clin Res, 10(11),* 16-19.

(iii) **Sanusi, S. B.,** Bakar, M. F. A., Mohamed, M., Sabran, S. F., and Isha, A. (2017). Preliminary phytochemical and anti-mycobacterial investigation of some selected medicinal plants of Endau Rompin, Johor, Malaysia. (Accepted for publication in Journal of Science and Technology).

(iv) **Sanusi, S. B.,** Bakar, M. F. A., Mohamed, M., Sabran, S. F., Norazlimi, N. A., and Isha, A. (2018). Effect of *Nepenthes ampularia* Jack extracts on the cell growth, cell membrane integrity and morphology of Mycobacterial cells. Submitted to International Journal of Microbiology.

(v) **Sanusi, S. B.,** Bakar, M. F. A., Mohamed, M., Sabran, S. F., Norazlimi, N. A., and Isha, A. Anitimycobacterial activity and Potential mechanism of action of Campnosperma auriculatum shoot extract. Submitted to Pharmaceutical Sciences Journal.

Conferences

(i) **Sanusi, S. B.,** Bakar, M. F. A., Mohamed, M., Sabran, S. F., and Isha, A. (2017). Preliminary phytochemical and anti-mycobacterial investigation of

some selected medicinal plants of Endau Rompin, Johor, Malaysia. The 3rd International Conference on the Application of Science and Mathematics, SCIEMATHIC2017. Organized by Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia (UTHM), Pagoh Educational Hub, 84600 Pagoh, Johor, Malaysia on 24-25th October 2017.

(ii) **Sanusi, S. B.,** Bakar, M. F. A., Mohamed, M., Sabran, S. F., and Isha, A. (2017). Antimycobacterial and antibacterial activities of *Macaranga gigantea* stem methanolic extract and its GC-MS profiling. In the 5th International Conference on Biological Sciences. Faculty of Biology, Universitas Gadjah Mada Indonesia, 15-16, 2017.

(iii) **Sanusi, S. B.,** Bakar, M. F. A., Mohamed, M., Sabran, S. F., and Isha, A. (2017). Antibacterial activity and phytochemical analysis of Kembang semangkok (*Scaphium macropodum*) stem bark. In the 5th International Conference on Biological Sciences. Faculty of Biology, Universitas Gadjah Mada Indonesia, 15-16, 2017.

CHAPTER 1

INTRODUCTION

This thesis described the anti-tuberculosis potential of selected medicinal plants utilised by Jakun ethnic group, residing in Kampung Peta, Mersing, Johor, Malaysia in the treatment of TB and TB-like symptoms. This study was carried out by using standardised chemical procedures to extract the compounds from the selected medicinal plants. The extracts were bioassayed using standard antimycobacterial test against a model organism of TB strain, *M. smegmatis* mc^2 155. Furthermore, the effect of the selected plant extracts on mycobacterial cell at cellular level was investigated. The phytochemical profiling of the selected plant extracts identified some compounds that could be responsible for the antimycobacterial properties. The claim by Jakun people for using the selected plants in the treatment of TB and TB-like symptoms were scientifically validated.

1.1 Background of the research

Tuberculosis (TB) is a deadly and common infectious disease caused by a pathogenic bacterium known as *Mycobacterium tuberculosis*. This disease is endemic in every country in the world, and it is the number one human killer among bacterial diseases (Hunter *et al.,* 2006; Adaikkappan *et al.,* 2012; Akintola *et al.,* 2013). Approximately, two billion individuals or around one-third of the entire global population are said to be infected with TB (Ch'ng *et al.,* 2017). Unprecedented decision was made by World Health Organization (WHO) in 1993 to declare TB as a public health global emergency (Abdallah & Ali, 2012; Rennie *et al.*, 2011), which is the only disease to be declared a global emergency by WHO (Palomino, 2009).

Among adults in their most productive age, TB can be the most significant causes of mortality and morbidity (Liao *et al.,* 2012). In the year 2015 WHO reported that there were 10.4 million occurrences of TB, equal to 142 cases for every 100,000 individuals. Around 1.4 million mortalities due to TB among HIV-negative persons were documented in 2015. TB accounted for one out of ten causes of mortality globally and instigated more death in comparison to HIV/AIDS in 2015 (Almatar *et al.,* 2017; WHO, 2016). It has been reported that 22 countries have the higher global TB prevalence. Over 50% of entire TB incidence happens in 5 countries of the Asian region (Liao *et al.,* 2012), viz.; Bangladesh, Myanmar, India, Thailand and Indonesia (Tasnim *et al.,* 2012).

In spite of government policies on prevention and control of TB, the disease is still regarded as a public health predicament in Malaysia (Rafiza *et al.,* 2011). In 2001, it was rated as the second most contagious infectious disease in Malaysia (Jetan *et al.,* 2010). Aside from being a killer disease, TB is an expensive disease to treat as well, which can contribute to a significant economic blow to the country. The resurfacing of this dilemma can be accredited to the high entry of foreign workers from neighboring countries with high TB incidence including Indonesia, Myanmar and Bangladesh into the country (Rafiza *et al.,* 2011). Hence, the justification why TB could be referred to as "a disease without borders" in Malaysia (Nissapatorn *et al.*, 2007).

Modern chemotherapy is used as a treatment to combat TB including isoniazid, rifampicin, ethambutol, streptomycin, and pyrazinamide (Altaf *et al.,* 2010). However, these drugs have drawbacks of causing adverse side effects such as hepatitis, gastrointestinal discomfort, and hearing loss (Famewo, Clarke, & Afolayan, 2017). Besides, there is always a chance of "relapse TB" due to course discontinuation of medication within the first year of treatment. Consequently, this situation results in a more severe condition where the *Mycobacterium* develops resistance to the TB drugs (Adaikkappan *et al.,* 2012; Gupta & Bhakta, 2012). The TB resistance can be classified into two: the multidrug-resistant TB (MDR-TB), which does not respond to the first-line standard treatment, and the extensive drug-resistant TB (XDR-TB), which happens when there is resistance to second-line TB drugs. According to WHO report in 2012 on surveillance and response to MDR-TB and XDR-TB, an estimate of 310,000 MDR-TB cases occurred among pulmonary TB patients recorded in 2011, with 84 countries reporting at least one case of XDR-

TB (Robles-zepeda *et al.,* 2013; Shashidhar *et al.,* 2015). Furthermore, 450,000 individuals in 2012 developed MDR-TB globally, and 170,000 deaths were reported from it, even though over half of these cases were reported from Brazil, China, Russia, and India; the overall picture is somewhat still alarming. The challenges present a unique opportunity to start exploring new approaches to treating MDR-TB (Umesiri *et al.,* 2015).

Due to these disadvantages of synthetic drugs, the potential efficacy of traditional medicines has motivated the interest of scientists and health care providers to turn onto conventional medicinal products for the treatment of some chronic diseases, including the treatment of TB (Abd Jalil *et al.,* 2012). Hence, the urgent need arises towards the search of a component with a higher anti-TB activity, easy availability and less side effects (Adaikkappan *et al.,* 2012; Bueno-Sánchez *et al.,* 2009; Kirimuhuzya *et al.,* 2009). Medicinal plants offer great hope to overcome these needs due to their chemical diversity and their significant role in the drug sighting and development. These plants have been used extensively as pure compounds or as a crude material. Only a few plant species have been thoroughly investigated for their medicinal properties (Gupta *et al.,* 2010; Gemechu *et al.,* 2013; Kaur & Kaur, 2015). For long, plant-based medicines have been used traditionally to treat a variety of illnesses worldwide. Around 75% of the global populace relies on medicinal plants for its primary health care (Jamal *et al.,* 2011). The phytochemical study of some of these plants has yielded a number of active natural products, although very little species have been comprehensively explored for their medicinal properties. So far, few plants have been tested against mycobacteria, and a few plants showed anti-TB activity (Bueno-Sánchez *et al.,* 2009; Kaur & Kaur, 2015).

Malaysia's tropical rainforest is occupied with various flora including herbal plants. In Malaysia, there are around 14,500 species of flowering plants of which over 2000 possess different medicinal qualities and have high potential to be commercialised (Jamal *et al.,* 2011; Nazmul *et al.,* 2011; Ahmad & Othman, 2013).

1.2 Problem statement

Of all the infectious diseases, TB is one of the leading killers of adults in the world today. Treatment regimens available have drawbacks of causing adverse side effects

such as hepatitis, gastrointestinal discomfort, nausea, and hearing loss. More so, the duration of the treatment is too long, taking 6-9 months for complete medication. Because of this reason, many individuals especially the rural inhabitants do not adhere to the prescribed duration for comprehensive treatment. Consequently, this situation results in a more severe condition where the *Mycobacterium* develops resistance to the TB drugs given rise to multidrug-resistant TB (MDR-TB) and extensive drug-resistant TB (XDR-TB). On the other hand, there are claims by traditional healers that the selected medicinal plants are used in the treatment of TB and TB related, but there is no study reported the scientific evaluation of the mycobacterial cells in these plants to justify the claim. As such, a laboratory screening of these plants needs to be carried out. Hence, the need for this study to determine and evaluate the anti-tuberculosis effects of medicinal plants. Researches have been done on anti-mycobacterial agents derived from natural products especially plants elsewhere in the world, however despite Malaysia being rich in plant diversity, very little attention has been given to the laboratory evaluation and detection of anti-mycobacterial activity from Malaysian medicinal plants.

1.3 Research objectives

This research aims to search for anti-TB potential from plants of Taman Negara Johor Endau-Rompin, exploring the traditional medical practices of the indigenous people of Kampung Peta (Jakun ethnic group).

In view of the aforementioned problems, this research embarks on the following objectives:

i. To determine the anti-mycobacterial activity of selected medicinal plant using *in vitro* assay.

ii. To investigate the effect of the extracts on mycobacterial cell at cellular level using time-kill assay, membrane integrity and cell damage (FESEM).

iii. To identify the phytochemical compounds present in the selected crude extracts potentially contributing to anti-mycobacterial activity.

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